DRAFT: August 31, 1994

DECISION DOCUMENT
TSCA SECTION 5(H)(4) EXEMPTION FOR
BACILLUS LICHENIFORMIS

I. SUMMARY

Bacillus licheniformis is a saprophytic bacterium which is widespread in nature and thought to contribute substantially to nutrient cycling due to the diversity of enzymes produced by members of the species. It has been used in the fermentation industry for production of enzymes, antibiotics and other specialty chemicals for over a decade with no known reports of adverse effects to human health or the environment. This species is easily differentiated from other members of the genus that are pathogenic to humans and animals. There are several reports in the literature of human infections with B. licheniformis, however, these occurred in immunosuppressed individuals or following trauma. There are no indications that B. licheniformis is pathogenic to plants. However, there are numerous reports in the literature of an association between B. licheniformis and abortions in livestock. In most reports, there were predisposing factors which may have resulted in immunosuppression of the affected animals. Since B. licheniformis is ubiquitous in the environment and appears to be an opportunistic pathogen in compromised hosts, the potential hazards associated with the use of this bacterium in fermentation facilities are low.

II. BACKGROUND

A. Introduction

EPA recognizes that some microorganisms present a low risk when used under specific conditions at general commercial use. Therefore, EPA is proposing expedited regulatory processes for certain microorganisms under these specific conditions at the general commercial use stage. Microorganism uses that would be exempt meet criteria addressing: (1) performance based standards for minimizing the numbers of microorganisms emitted from the manufacturing facility; (2) the introduced genetic material; and (3) the recipient microorganism. Microorganisms that qualify for these exemptions, termed Tier I and Tier II, must meet a standard of no unreasonable risk in the exempted use.

To evaluate the potential for unreasonable risk to human health or the environment in developing these exemptions, EPA focuses primarily on the characteristics of the recipient

microorganisms. If the recipient is shown to have little or no potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is also specifying procedures for minimizing numbers of organisms emitted from the facility. When balanced against resource savings for society and expected product benefits, these exemptions will not present unreasonable risks.

B. Criteria for Minimizing Release from Manufacturing Facilities

The standards prescribed for the Tier I exemption require the following: (1) the structure(s) be designed and operated to contain the microorganism, (2) access to the structure should be limited to essential personnel, (3) inactivation procedures shown to be effective in reducing the number of viable microorganisms in liquid and solid wastes should be followed prior to disposal of the wastes, (4) features to reduce microbial concentrations in aerosols and exhaust gases released from the structure should be in place, and (5) general worker hygiene and protection practices should be followed.

1. Definition of structure. EPA considers the term "structure" to refer to the building or vessel which effectively surrounds and encloses the microorganism. Vessels may have a variety of forms, e.g., cubic, ovoid, cylindrical, or spherical, and may be the fermentation vessel proper or part of the downstream product separation and purification line. All would perform the function of enclosing the microorganism. In general, the material used in the construction of such structure(s) would be impermeable, resistant to corrosion and easy to clean/sterilize. Seams, joints, fittings, associated process piping, fasteners and other similar elements would be sealed.

2. Standards to minimize microbial release. EPA is proposing, for several reasons, a somewhat cautious approach in prescribing standards for minimizing the number of microorganisms emitted through the disposal of waste and the venting of gases. First, a wide range of behaviors can be displayed by microorganisms modified consistent with EPA's standards for the introduced genetic material. Second, EPA will not conduct any review whatsoever for Tier I exemptions. EPA believes the requirement to minimize emissions will provide a measure of risk reduction necessary for making a finding of no unreasonable risk. Taken together, EPA's standards ensure that the number of microorganisms emitted from the structure is minimized.

EPA's proposed standards for minimizing emissions specify that liquid and solid waste containing the microorganisms be treated to give a validated decrease in viable microbial populations so that at least 99.9999 percent of the organisms resulting from the fermentation will be killed. Since the bacteria used in fermentation processes are usually debilitated, either intentionally or through acclimation to industrial fermentation, the small fraction of microorganisms remaining viable after inactivation treatments will likely have a reduced ability to survive during disposal or in the environment. Moreover, industrial companies, in an attempt to keep their proprietary microorganisms from competitors and to reduce the microbial numbers to those permitted by local sanitation authorities, modify the microorganisms to increase the ability of their microorganisms to survive and perform their assigned tasks in the fermentor but decrease their ability to survive in the environment external to the fermentor.

EPA requirements also address microorganisms in the exhaust from the fermentor and along the production line. To address exhaust from fermentors, EPA is proposing that the number of microorganisms in fermentor gases be reduced by at least two logs prior to the gases being exhausted from the fermentor. EPA selected this number based on an estimate of the numbers of microorganisms likely to be in the exhaust from an uncontrolled fermentor and common industry practice. Moreover, microorganisms that are physiologically acclimated to the growth conditions within the fermentor are likely to be compromised in their ability to survive aerosolization. EPA anticipates, therefore, that few microorganisms will survive the stresses of aerosolization associated with being exhausted in a gas from the fermentor. The provision requiring reduction of microorganisms in fermentor exhaust gases contributes to minimizing the number of viable microorganisms emitted from the facility.

EPA is also proposing that the requirements specify that other systems be in place to control dissemination of microorganisms by other routes. This would include programs to control pests such as insects or rats, since these might serve as vectors for carrying microorganisms out of the fermentation facilities.

3. Worker protection. The requirement to minimize microbial emissions, in conjunction with the requirement for general worker safety and hygiene procedures, also affords a measure of protection for workers. Potential effects on workers that exist with microorganisms in general (e.g., allergenicity) will be present with the microorganisms qualifying for this exemption. As with other substances that humans may react to

(e.g., pollen, chemicals, dust), the type and degree of allergenic response is determined by the biology of the exposed individual. It is unlikely that a microorganism modified in keeping with EPA's specifications for the introduced genetic material would induce a heightened response. The general worker hygiene procedures specified by EPA should protect most individuals from the allergenic responses associated with microorganisms exhausted from fermentors and/or other substances emitted along the production line. The EPA requirement that entry be limited to essential personnel also addresses this consideration by reducing to a minimum the number of individuals exposed.

4. Effect of containment criteria. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption. EPA is not specifying standards for minimizing the number of microorganisms emitted from the facility for microorganisms qualifying for Tier II exemption. Rather, the Agency requests that submitters utilize as guidance the standards set forth for Tier I procedures. The procedures proposed by the submitter in a Tier II exemption request will be reviewed by the Agency. EPA will have the opportunity to evaluate whether the procedures the submitter intends to implement for reducing the number of organisms emitted from the facility are appropriate for that microorganism.

C. Introduced Genetic Material Criteria

In order to qualify for either Tier I or Tier II exemption, any introduced genetic material must be limited in size, well characterized, free of certain nucleotide sequences, and poorly mobilizable.

1. Limited in size. Introduced genetic material must be limited in size to consist only of the following: (1) the structural gene(s) of interest; (2) the regulatory sequences permitting the expression of solely the gene(s) of interest; (3) the associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites; (4) the nucleotide sequences needed for vector transfer; and (5) the nucleotide sequences needed for vector maintenance.

The limited in size criterion reduces risk by excluding the introduction into a recipient of extraneous and potentially uncharacterized genetic material. The requirement that the regulatory sequences permit the expression solely of the structural gene(s) of interest reduces risk by preventing

expression of genes downstream of the inserted genetic material. The limitation on the vector sequences that are components of the introduced genetic material prevents the introduction of novel traits beyond those associated with the gene(s) of interest. The overall result of the limited in size criterion is improved ability to predict the behavior of the resulting microorganism.

2. Well characterized. For introduced genetic material, well characterized means that the following have been determined: (1) the function of all of the products expressed from the structural gene(s); (2) the function of sequences that participate in the regulation of expression of the structural gene(s); and (3) the presence or absence of associated nucleotide sequences.

Well characterized includes knowledge of the function of the introduced sequences and the phenotypic expression associated with the introduced genetic material. Genetic material which has been examined at the restriction map or sequence level, but for which a function or phenotypic trait has not yet been ascribed, is not considered well characterized. Well characterized would include knowing whether multiple reading frames exist within the operon. This relates to whether more than one biological product might be encoded by a single sequence, and addresses the possibility that a modified microorganism could display unpredicted behavior should such multiple reading frames exist and their action not be anticipated.

3. Free of certain sequences. In addition to improving the ability to predict the behavior of the modified microorganism, the well characterized requirement ensures that segments encoding for either part or the whole of the toxins listed in the proposed regulatory text for the TSCA biotechnology rule would not inadvertently be introduced into the recipient microorganism.

These toxins are polypeptides of relatively high potency. Other types of toxins (e.g., modified amino acids, heterocyclic compounds, complex polysaccharides, glycoproteins, and peptides) are not listed for two reasons. First, their toxicity falls within the range of moderate to low. Second, these types of toxins generally arise from the activity of a number of genes in several metabolic pathways (multigenic).

In order for a microorganism to produce toxins of multigenic origin, a large number of different sequences would have to be introduced and appropriately expressed. It is unlikely that all of the genetic material necessary for metabolizing multigenic toxins would be inadvertently introduced into a recipient

microorganism when requirements that the genetic material be limited in size and well characterized are followed.

Similarly, other properties that might present risk concerns result from the interactive expression of a large number of genes. For example, pathogenic behavior is the result of a large number of genes being appropriately expressed. Because of the complex nature of behaviors such as pathogenicity, the probability is low that an insert consisting of well characterized, limited in size genetic material could transform the microorganisms proposed for exemption into microorganisms which display pathogenic behavior.

4. Poorly mobilizable. Poorly mobilizable means the ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of transfer of less than 10^{-8} transfer events per recipient. The requirement that the introduced genetic material be poorly mobilizable reduces potential for transfer of introduced genetic sequences to other microorganisms in the environment. Such transfers would occur through the interaction of the introduced microorganism with indigenous microorganisms through conjugation, transduction, or transformation. Through such transfers, the introduced genetic material could be transferred to and propagated within different populations of microorganisms, including microorganisms which may never previously have been exposed to this genetic material. It is not possible to predict how the behavior of these potential recipient microorganisms will be affected after uptake and expression of the genetic material.

Since EPA is not limiting the type of organism that can serve as the source for the introduced genetic material, some limitation is placed on the ability of the introduced genetic material to be transferred. This limitation mitigates risk by significantly reducing the probability that the introduced genetic material would be transferred to and expressed by other microorganisms.

The 10^{-8} frequency is attainable given current techniques. Plasmids with transfer rates of 10^{-8} exist or are easily constructed. Some of the plasmids most commonly employed as vectors in genetic engineering (e.g., pBR325, pBR322) have mobilization/transfer frequencies of 10^{-8} or less.

The criteria set for "poorly mobilizable" for transduction and transformation should not prevent most microorganisms from meeting the exemption criteria, since the majority of transfer frequencies reported for transduction and natural transformation are less than 10^{-8}. Higher frequencies are likely only if the

introduced genetic material has been altered or selected to enhance frequency.

Fungal gene transfer has also been considered in development of the poorly mobilizable criterion. Although mobile genetic elements such as transposons, plasmids and double stranded RNA exist in fungi and can be readily transferred, this transfer usually is only possible between members of the same species during anastomosis, a process specific to fungi. Since anastomosis only occurs between members of the same species, the introduced genetic material would not be transferred to distantly related fungi as may occur with bacteria.

5. Effect of introduced genetic material criteria. The requirements placed on the introduced genetic material, in concert with the level of safety associated with Bacillus licheniformis, ensure that the resulting microorganisms present low or negligible risk. The probability is low that the insertion of genetic material meeting EPA's criteria into strains of B. licheniformis will change their behavior so that they would acquire the potential for causing adverse effects. Risks would be mitigated by the four criteria placed on the introduced genetic material, the relative safety of B. licheniformis, and the inactivation criteria specified for the Tier I exemption. In the case of Tier II exemption, risks would be mitigated in light of the four criteria placed on introduced genetic material, the relative safety of B. licheniformis, and EPA's review of the conditions selected.

D. Recipient Microorganism Criteria

Six criteria were used by EPA to determine eligibility of recipient microorganisms for the tiered exemption. Microorganisms which EPA finds meet these criteria are listed as eligible recipients. The first criteria would require that it be possible to clearly identify and classify the microorganism. Available genotypic and phenotypic information should allow the microorganism to be assigned without confusion to an existing taxon which is easily recognized. Second, information should be available to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on human health or the environment. Third, there should be a history of commercial use for the microorganism. Fourth, the commercial uses should indicate that the microorganism products might be subject to TSCA jurisdiction. Fifth, studies are available which indicate the potential for the microorganism to cause adverse effects on human health and the environment. Sixth, studies are available which indicate the survival characteristics of the microorganism in the environment.

After each microorganism was reviewed using the six evaluation criteria, a decision was made as to whether to place the microorganism on the list. The Agency's specific determination for Bacillus licheniformis is discussed in the next unit.

III. EVALUATION OF BACILLUS LICHENIFORMIS

A. History of Use

1. History of safe commercial use. B. licheniformis has been used in the fermentation industry for over a decade for production of proteases, amylases, antibiotics, or specialty chemicals. The ATCC Catalogue of Bacteria and Phages lists strains which are capable of producing alkaline proteases, a-amylases, penicillinase, pentosanases, bacitracin, proticin, 5'-inosinic acid and inosine, citric acid, and substituted L-tryptophan. B. licheniformis is considered a Class 1 Containment Agent under the NIH Guidelines for Research Involving Recombinant DNA Molecules and also falls under Class 1 Containment under the European Federation of Biotechnology guidelines.

2. Products subject to TSCA jurisdiction. B. licheniformis is commonly used to produce proteases and a-amylase. Potential TSCA uses for proteases include dehairing and batting in the leather industry. Other TSCA uses of a-amylase include desizing of textiles and starch modification for paper sizing. To date, EPA has reviewed three premanufacture notices (PMNs) for strains of B. licheniformis. Two of these microorganisms were modified for enhanced production of the enzyme a-amylase to be used in dishwashing and laundry detergent formulations for starch breakdown and to be used in the textile industry for desizing of textiles prior to dyeing.

B. Identification of Microorganism

1. Classification. The genus Bacillus consists of a large number of diverse, rod-shaped gram positive bacteria which are capable of producing endospores that are resistant to adverse environmental conditions. Recent work has suggested that B. licheniformis is one of the better defined bacillus species. The species is genetically homogeneous based on DNA-DNA hybridization studies. B. licheniformis can be distinguished from other Bacillus species by the use of API diagnostic test kits or pyrolysis gas-liquid chromatography.

2. Related taxa of concern. B. licheniformis is part of the same large cluster of bacilli which includes pathogenic or opportunistic Bacillus species. This includes the B. cereus/anthracis/thuringiensis/mycoides group whose members are mammalian and insect pathogens and food poisoning agents. However, B. licheniformis is distinguishable from the pathogenic bacilli as well as from the more closely related bacilli, B. subtilis and B. pumilus.

C. Risk Summary

1. Studies regarding potential for adverse effects. B. licheniformis is not a frank human pathogen, but has been isolated from human infections. However, the literature suggests that before infection can occur, there must be immunosuppression of the host or trauma followed by inoculation in high numbers. B. licheniformis does not produce significant quantities of extracellular enzymes or toxins and is generally considered to have a low degree of virulence.

The literature also indicates that ecological hazards associated with the use of B. licheniformis are low. While there are reports suggesting that B. licheniformis is a cause of abortion in livestock, Koch's postulates have not been satisfied in demonstrating that this microorganism was the causal agent. The association of B. licheniformis with livestock abortions is quite low compared to the total number of livestock abortions caused by microorganisms.

Due to its ubiquitous presence as spores in soil and dust, B. licheniformis is widely known as a contaminant of food; however, it is not typically considered to be a causal agent of food poisoning. No reports in the literature suggest that B. licheniformis is a plant pathogen. While B. licheniformis is capable of producing several antimicrobial compounds in culture, the importance of antibiotic production in the soil community is unknown. B. licheniformis has been recently investigated for its use for biocontrol of several pathogenic fungi.

2. Studies regarding survival in the environment. B. licheniformis is ubiquitous in nature, existing predominantly in soil as spores. Unlike other bacilli which are typically aerobic, B. licheniformis is facultatively anaerobic, allowing for growth in additional ecological niches. The microorganism is usually saprophytic. Its production of proteases and ability to break down complex polysaccharides enables it to contribute substantially to nutrient cycling. While certain members of the species are capable of denitrification, their contribution to

bacterial denitrification would be small because they typically persist in soil as endospores.

IV. BENEFITS SUMMARY

Substantial benefits are associated with this proposed exemption. Bacillus licheniformis is already widely employed in general commercial uses, some of which are subject to TSCA reporting. The Agency believes this exemption will result in resource savings both to EPA and industry without compromising the level of risk management afforded by the full 90 day review. In addition to assessing the risk of B. licheniformis, EPA has developed criteria limiting the potential for transfer of and expression of toxin sequences, and the conditions of use specified in the exemption are met (Tier I) or will be reviewed by EPA to ensure adequate risk reduction (Tier II). EPA requirements for minimizing numbers of viable microorganisms emitted are within standard operating procedures for the industry, and both the procedures and the structures specified in the exemption are the type industry uses to protect their products from contamination.

The exemption will result in reduced reporting costs and a decrease in delay associated with reporting requirements. The savings in Agency resources can be directed to reviewing activities and microorganisms which present greater uncertainty. This exemption should also facilitate development and manufacturing of new products and the accumulation of useful information.

V. RECOMMENDATION AND RATIONALE

A. Recommendation: Bacillus licheniformis is recommended for the TSCA section 5(h)(4) exemption.

B. Rationale

1. Risks from use of the recipient microorganism B. licheniformis are low. B. licheniformis is ubiquitous in the environment and the releases expected from fermentation facilities will not significantly increase populations of this microorganism in the environment. Although the possibility of human infection by B. licheniformis is not non-existent, it is low in the industrial setting, because it occurs primarily in highly immunocompromised individuals. Infection might be a possibility following trauma, but in the industrial setting with the use of proper safety precautions, good laboratory practices,

and proper protective clothing and eyewear, the potential for infection of workers should be quite low. Although B. licheniformis may be associated with livestock abortions, the use of this microorganism in fermentation facilities will not substantially increase the frequency of this occurrence.

2. Use of strains of B. licheniformis which are eligible for the TSCA section 5(h)(4) exemption present no unreasonable risk. While not completely innocuous, B. licheniformis presents low risk of adverse effects to human health or the environment. Because the recipient microorganism was found to have little potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption and will be reviewing the conditions selected for the Tier II exemption. When balanced against resource savings for society and expected product benefits, this exemption will not present unreasonable risks.

REQUEST FOR COMMENTS

The Risk Assessment to support the proposal of Bacillus licheniformis as a candidate for the TSCA section 5(h)(4) tiered exemption recommends that only asporogenic strains with a sporulation deficiency of at least 10^{-7} be eligible for the exemption. However, this Decision Document recommends all strains of Bacillus licheniformis for this exemption. The recipient microorganism B. licheniformis was found to have little potential for adverse effects. The probability is low that the insertion of genetic material meeting EPA's criteria into such a microorganism will change its behavior so that it would acquire the potential for causing adverse effects. Therefore, there should be no need to restrict this exemption to asporogenic strains.

However, because there is a discrepancy in the recommendations of the Risk Assessment and the Decision Document, EPA requests comment on whether its current recommendation of all strains of B. licheniformis as eligible for this exemption is appropriate or should be modified to limit the exemption only to asporogenic strains.

Attachment 1:

INTEGRATED RISK ASSESSMENT OF

BACILLUS LICHENIFORMIS

I. INTRODUCTION

Bacillus licheniformis is a saprophytic bacterium that is widespread in nature and thought to contribute substantially to nutrient cycling due to the diversity of enzymes produced by members of the species. It has been used in the fermentation industry for production of proteases, amylases, antibiotics, and specialty chemicals for over a decade with no known reports of adverse effects to human health or the environment. This species is easily differentiated from other members of the genus that are pathogenic to humans and animals. There are several reports in the literature of human infections with *B. licheniformis*, however, these occurred in immunosuppressed individuals or following trauma. There are no indications that *B. licheniformis* is pathogenic to plants. However, there are numerous reports in the literature of an association between *B. licheniformis* and abortions in livestock. In most reports, there were predisposing factors which may have resulted in immunosuppression of the affected animals. Since *B. licheniformis* is ubiquitous in the environment and appears to be an opportunistic pathogen in compromised hosts, the potential risk associated with the use of this bacterium in fermentation facilities is low.

History of Commercial Use and Products Subject to TSCA Jurisdiction

B. licheniformis has been used in the fermentation industry for over a decade for production of proteases, amylases, antibiotics, or specialty chemicals. The ATCC Catalogue of Bacteria and Phages lists strains which are capable of producing alkaline proteases, alpha-amylases, penicillinases, pentosanases, bacitracin, proticin, 5'-inosinic acid and inosine, citric acid, and substituted L-tryptophan (Gherna et al., 1989). Statistics from ten years ago (Eveleigh, 1981), indicated that industrial microbial fermentation was responsible for production of 530 tons of protease and 320 tons of alpha-amylase on an annual basis. According to Eveleigh (1981), the main industrial protease was one produced by *B. licheniformis* for use as a cleaning aid in detergents. Other TSCA uses for proteases include dehairing and batting in the leather industry and TSCA uses of alpha-amylase

include desizing of textiles and starch modification for sizing of paper (Erikson, 1976).

EPA has reviewed, under TSCA, a genetically modified strain of *B. licheniformis* used for the production of a hydrolase enzyme (P87-1511), and two recombinant strains for production of alpha-amylase (P89-1071, and P92-50).

II. IDENTIFICATION AND TAXONOMY

A. Overview

Bacillus licheniformis is a ubiquitous bacterium thought to be of importance in the environment as a contributor to nutrient cycling due to the production of protease and amylase enzymes (Claus and Berkeley, 1986). Although the actual numbers in existence in the environment for this species have not been determined, in general, bacilli occur at population levels of 10^6 to 10^7 per gram of soil (Alexander, 1977). *B. cereus* is isolated most frequently from soils; however, this is thought to be due to its ability to crowd-out other species in enrichment culture rather than reflecting an actual predominance in soils (Norris et al., 1981). Unless a soil has been recently amended with organic matter which provides for readily utilizable nutrients for vegetative cells, the bacilli exist predominately as endospores. It is thought that between 60 to 100 % of soil populations of *Bacillus* exist in the inactive spore state and that these endospores are capable of surviving for many years (Alexander, 1977).

B. Taxonomy and Characterization

The genus *Bacillus* consists of a large number of diverse, rod-shaped Gram positive (or positive only in early stages of growth) bacteria which are capable of producing endospores that are resistant to adverse environmental conditions such as heat and desiccation (Claus and Berkeley, 1986). Typically, the cells are motile by peritrichous flagella and are aerobic. The genus consists of a diverse group of organisms as evidenced by the wide range of DNA base ratios of approximately 32 to 69 mol% G + C (Claus and Berkeley, 1986) which is far wider than usually considered reasonable for a genus (Norris et al., 1981).

B. licheniformis is ubiquitous in nature, existing predominately in soil as spores. Unlike other bacilli that are typically aerobic, *B. licheniformis* is facultatively anaerobic, allowing for growth in additional ecological niches. The microorganism is usually saprophytic. Its production of

proteases and ability to break down complex polysaccharides enables it to contribute substantially to nutrient cycling (Claus and Berkeley, 1986). Certain members of the species are capable of denitrification; however, their importance in bacterial denitrification in the environment is considered to be small as the bacilli typically persist in soil as endospores (Alexander, 1977).

The *Bacillus* species *subtilis*, *licheniformis*, and *pumulis* are closely related, and historically, there has been difficulty distinguishing among the three species. Gordon (1973), who conducted much of the pioneering work on the taxonomy of the genus, referred to these three species as the subtilis-group or subtilis-spectrum.

More recent work has suggested that *B. licheniformis* is one of the better defined *Bacillus* species. The species is genetically homogeneous based on DNA-DNA hybridization studies (Claus and Berkeley, 1986). In addition, Seki et al. (1975) demonstrated that DNA-DNA hybridization studies correlated well with species identification using conventional taxonomic characteristics such as those in Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1986). Based on numeric taxonomic analyses, Priest et al. (1988) placed *B. licheniformis* in a unique phenotypic cluster positioned close to, and between, *B. subtilis* and *B. pumilus*. Independently, similar but unpublished work done for EPA by the Microbial Systematics Section at the National Institute of Dental Research provided a tight cluster of *B. licheniformis* strains. As in the Priest et al. (1988) study, most strains clustered at the 92% level, but strains at the edges overlapped into the adjacent cluster, a small group of *B. pumilus*. Two *B. pumilus* strains also were embedded in the *B. licheniformis* portion of the identification matrix. However, other studies have shown that *B. licheniformis* could be fairly readily differentiated from other species in the genus by the use of API diagnostic test kits (Logan and Berkeley, 1981). In addition, *B. licheniformis* was also easily distinguishable from other closely related members of the genus using pyrolysis gas-liquid chromatography (O'Donnell et al., 1980.)

C. Related Species of Concern

There are several species of the genus which are known pathogens. These include *B. anthracis* which is pathogenic to humans and other animals, and *B. cereus* which is a common cause of food poisoning (Claus and Berkeley, 1986; Norris et al., 1981). *B. thuringiensis*, *B. larvae*, *B. lentimorbus*, *B. popilliae*, and some strains of *B. sphaericus* are pathogenic to

certain insects. Other species in the genus can be opportunistic pathogens of humans or animals.

In a numerical classification using 118 characteristics of 368 species of *Bacillus*, the species *B. thuringiensis, B. cereus*, and *B. mycoides* clustered together at 89 - 92% similarity (Priest et al., 1988). The *B. subtilis* group, to which *B. licheniformis* belongs, joined the *B. cereus* group at 72% relatedness. Therefore, there is no difficulty in distinguishing between the toxin-producing strains of *Bacillus* and *B. licheniformis*.

III. HAZARD ASSESSMENT

A. Human Health Hazards

1. Colonization

Bacillus licheniformis is a ubiquitous organism and likely enters the human digestive system many times a day. While data regarding its ability to survive in the human gastrointestinal tract are sparse, it is likely that the spores will pass through without causing harm. Outside the gastrointestinal tract, the organism would likely be a temporary inhabitant of skin. Although it can grow over a wide range of temperatures including that of the human body (Claus and Berkeley, 1986), it is unlikely that this microorganism will colonize humans to any large degree. Contact with the microorganism, therefore, would generally be relegated to soil and other environmental sources.

2. Gene Transfer

While the species itself does not appear to have virulence factor genes, the genus *Bacillus* is known to be able to acquire plasmids from other bacteria in the environment. There is evidence to suggest that other species of *Bacillus*, such as *B. subtilis,* actively exchange genetic information in the soil (Graham and Istock, 1979). It is, therefore, theoretically possible for *B. licheniformis* to acquire the ability to produce toxins or other virulence factors; however, this has not been demonstrated.

3. Toxin Production

A review of the literature by Edberg (1992) failed to reveal toxigenic substances produced by *B. licheniformis*. While there have been cases of acute, self-limited gastroenteritis associated with the isolation of large numbers of this species, a toxic or

direct effect on intestinal epithelia has not been demonstrated. It is difficult to ascertain whether the species in these reported cases, which are quite limited in number, actively participated in the infection or were isolated in conjunction with an unidentified pathogen. Obi (1980) reported that a number of species of the genus *Bacillus*, including *B. licheniformis, B. subtilis, B. megaterium,* and *B. pumilus,* were able to produce a lecithinase. Lecithinase is an enzyme that can disrupt the cell membrane of mammalian cells. However, there has not been a correlation with production of this lecithinase and human disease.

4. Measure of the Degree of Virulence

While not innocuous, *B. licheniformis* appears to have a very low degree of virulence. It does not produce significant quantities of extracellular enzymes and other factors likely to predispose it to cause infection. The species has been isolated a number of times from human infections. The literature (cited below) suggests that there must be immunosuppression or trauma in order for infection with this species to occur. Farrar (1963) divided human infections by species of *Bacillus* into the following groups: (1) local infections of a closed space, such as the eye, in which the organism is inoculated in high numbers secondary to trauma, (2) mixed infections in which the species of *Bacillus* is found in the company of other organisms with higher virulence properties, and (3) disseminated infections, usually in profoundly immunosuppressed individuals, in which the species is recovered from multiple sites, usually including the blood stream.

Reviews of *Bacillus* infections from several major hospitals have indicated the relative lack of virulence of *B. licheniformis.* For example, Ihde and Armstrong (1973) reviewed cases at Memorial Sloan Kettering Cancer Hospital over a 6-1/2 year period. Unidentified species of *Bacillus* were isolated in twelve cases of infection, two of which were felt to be serious. Banerjee et al. (1988), reviewing all *Bacillus* bacteremia cases during a six-year period from 1978 to 1986, found 18 febrile patients experiencing 24 episodes of bacteremia. *B. licheniformis* was isolated from one case. Of these 18 patients, 15 had lymphoma or leukemia and three had breast cancer. Nine of the patients had neutrophil counts of less than 1000. Seven of these patients had an indwelling Hickman catheter in place. Scanning and transmission electron microscopy from one of the Hickman catheters showed *Bacillus* organisms growing in a biofilm inside the Hickman catheter. By comparison, during the same period, there were 1,038 cases of bacteremia.

In a review article, Logan (1988) reported several infections produced by *B. licheniformis*. One case was an ophthalmitis, a corneal ulcer, following trauma (Tabbara and Tarabay, 1979). Other cases included septicemia and bacteremia, and peritonitis with bacteremia in a patient with an upper small bowel perforation (Sugar and McCloskey, 1977). In the literature, there is also circumstantial evidence implicating *B. licheniformis* as a cause of food poisoning (Gilbert et al., 1981; Kramer et al., 1982). Fuchs et al. (1984) and Pessa et al. (1985) described *Bacillus* infections associated with intravenous catheters.

In a 10 year review of records at the Yale-New Haven Hospital, *B. licheniformis* was isolated four times as a cause of infection (Edberg, 1992). In two patients the species was associated with eye trauma; in one patient it was associated with a silicone-based implant; and in the fourth patient it was associated with metastatic lung cancer.

5. Overall Assessment of Virulence

Edberg (1992) concluded that the virulence characteristics of *B. licheniformis* are very low. He stated that in order to achieve an infection, either the number of microorganisms must be very high or the immune status of the host low. While the possibility of infection with *B. licheniformis* is low, it is not non-existent.

6. Other Hazards

Due to its ubiquitous presence as spores in soil and dust, *B. licheniformis* is widely known as a contaminant of food (Norris et al., 1981). It is a common spoilage organism of milk (Mostert et al., 1979; Foschino et al., 1990), packaged meats (Bell and DeLacy, 1984), and some canned goods (Norris et al., 1981). However, it is typically not thought to be a causal agent of food poisoning. *B. licheniformis* has also been shown to be a contaminant of pharmaceutical tablets (Nandapurkar et al., 1985.)

7. Conclusions

B. licheniformis is not a human pathogen nor is it toxigenic. It is unlikely to be confused with related species that are. However, if challenged by large numbers of this microorganism, compromised individuals or those suffering from trauma may be infected.

B. Environmental Hazards

1. Hazards to Animals

There are numerous reports in the literature on the association of *B. licheniformis* with livestock abortions (for a more detailed account, see McClung, 1992). In a recent review article, Logan (1988) stated that isolations of *B. licheniformis* from bovine and ovine abortions appear quite regularly in the Veterinary Record by the Veterinary Investigation Service and the Scottish Investigation Service, especially after wet summers when the silage is of low quality. Ryan (1970) reported the isolation of *B. licheniformis* in two cases of cattle abortion. Although it was not possible to attribute this microorganism as the causal agent, attempts to demonstrate other infectious agents yielded negative results. Likewise, Mitchell and Barton (1986) also reported isolation of only *B. licheniformis* in three cases of bovine abortion. The presence of the *B. licheniformis* in fetal stomach contents suggests that the bacterium is capable of entering the bloodstream of the adult animals and crossing the placenta to the fetus.

Johnson et al. (1983) reported the death of 15 calves due to *B. licheniformis* infection in a herd in Scotland. In all cases, no viruses or bacteria other than *B. licheniformis* were isolated from the stomach contents and internal organs. However, this herd apparently was debilitated by (1) an earlier infection with BVD (bovine viral diarrhea) virus which is known to cause immunosuppression in cattle, and (2) a severe vitamin A deficiency from poor quality, moldy hay. The authors speculated that the feeding for three months on poor quality hay had exposed the calves to a heavy challenge of *B. licheniformis* both through ingestion and inhalation.

According to a veterinary diagnostician in this country, the incidence of bovine abortion caused by members of the *Bacillus* genus (both *B. licheniformis* and *B. cereus* grouped together) was 3.5% of the total abortions and stillbirths examined (8,962) over a 10-year period in South Dakota (Kirkbride, 1993). The total number of abortions and stillbirths caused by all bacteria was 14.49%. *Bacillus* ranked second in frequency of occurrence, after *Actinomyces pyrogenes*. The fact that abortions associated with *Bacillus* species are less common compared to other microorganisms, particularly viruses and fungi, has resulted in very little research being conducted to investigate whether *B. licheniformis* is the actual causal agent in these cases. The veterinary diagnostics laboratories in this country make attempts to isolate any and all microorganisms present in the aborted fetuses which are sent to them for inspection. However, there is no determination of whether the organism(s) isolated are the etiological agents and often there is little background

information supplied as to whether there were predisposing factors which may have led to compromised immune systems in the animals.

B. licheniformis has also been reported to be associated with abortions in swine (Kirkbride et al., 1986). Members of the genus *Bacillus* have also been associated with abortions in sheep (Mason and Munday, 1968; Smith and Frost, 1968); however, in both these latter reports, species identification was not made.

There are also reports in the literature of associations of *B. licheniformis* with bovine mastitis (Logan, 1988) and goat mastitis (Kalogridou-Vassiliadou, 1991).

In addition, Wright et al. (1978) reported a water-borne *B. licheniformis* infection in laboratory mice which resulted in depressed hemoglobin content, white cells and platelet counts.

Many of the reports on livestock abortion have suggested that *B. licheniformis* is a causal agent. This has been shown to be the case for *B. cereus* where inoculation of the microorganism resulted in cattle abortion (Wohlgemuth et al., 1972). As yet, no one has confirmed *B. licheniformis* as the actual etiological agent in animal abortions. This literature also suggests that in these cases of *B. licheniformis* infection, the livestock was in a compromised immune state. According to Kirkbride (1993), the immune reaction at the junction of the maternal and fetal placentas is suppressed, most likely to prevent rejection of the fetus. Consequently, opportunistic microorganisms, even with low virulence, have the ability to multiply and cause lesions, and result in abortion.

2. Hazards to Plants

No reports in the literature were encountered that suggested that *B. licheniformis* is a plant pathogen. There was no mention of any plant pathogenic activity in Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1986) nor in the U.S. Department of Agriculture list of pathogens under the Federal Plant Pest Act (7 CFR 330, et seq.).

3. Hazards Posed to Other Microorganisms

B. licheniformis is capable of producing several antimicrobial compounds. It produces the antibiotics licheniformin (Callow and Hart, 1946), bacitracin (Johnson et al., 1945), and at least one other antibiotic from a certain strain, 2725 (Woolford, 1972). Bacitracin is active mainly against Gram positive bacteria, whereas the antibiotic from

strain 2725 is active against various Gram positive and Gram negative species (Woolford, 1972). These antibiotics have been shown to be produced in culture, however, the importance of antibiotic production in regulating the soil community and the significance in the environment is unknown (Alexander, 1977).

B. licheniformis has been shown to be inhibitory to the growth of various fungi and has recently been investigated for its use as a biocontrol agent of several fungal pathogens. Shigemitsu et al. (1983) noted malformation of *Fusarium oxysporum* f. sp. *cucumerinum* caused by metabolite(s) produced by *B. licheniformis* when the organisms were cultured together. Scharen and Bryan (1981) also showed that metabolites of *B. licheniformis* produced in culture were antagonistic to *Pyrenophora teres*, the cause of net blotch of barley. When applied to the leaves of barley seedlings, *B. licheniformis* established itself and prevented infection by the fungus. Likewise, *B. licheniformis* was shown to be antagonistic to *Pyrenophora tritici-repentis* which causes wheat tan spot (Mehdizadegan, 1987). Singh and Dwivedi (1987) reported that *B. licheniformis* reduced the growth of *Sclerotium rolfsii* sacc. (the causal agent of foot rot of barley) by 31% in mixed culture. The metabolites alone produced by the bacilli in culture were also inhibitory to the pathogen. In addition, *B. licheniformis* was shown to be antagonistic to *Phymatotrichum omnivorum*, the cause of cotton root rot (Cook et al., 1987). Although *B. licheniformis* and/or products produced by the microorganism are inhibitory to the growth of numerous other microorganisms in the environment, due to the widespread nature of this bacterium, it is unlikely that any perturbations in microbial community structure would occur by the potential release of additional numbers of these microorganisms to the environment from fermentation facilities operating under the conditions of the exemption.

4. Conclusions

The issue of livestock abortions is the most serious environmental hazard identified for *B. licheniformis*. However, it has not been scientifically established that *B. licheniformis* is the causative agent. *B. licheniformis* appears to be an opportunistic pathogen that may create problems in immunocompromised livestock. However, livestock abortions associated with *Bacillus* species are infrequent compared to other microorganisms.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

B. licheniformis is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986). This microorganism also falls under the Class 1 Containment under the European Federation of Biotechnology guidelines (Frommer et al., 1989).

No data were available for assessing the release and survival specifically for fermentation facilities using *B. licheniformis*. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, ie. near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day.

Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

B. licheniformis is a common saprophytic inhabitant of soils and is capable of producing endospores when vegetative growth conditions are unfavorable. Unlike most bacilli, growth occurs under anaerobic conditions as well as aerobic, and occurs at temperatures as high as 55C (Claus and Berkeley, 1986). The endospores produced by *B. licheniformis* resist severe heat treatment (Claus and Berkeley, 1986). Specific data comparing the survivability of industrial and wild-type strains of *B. licheniformis* were not available in the existing literature. However, the ability of *B. licheniformis* to produce highly resistant spores and grow under a wide range of conditions indicates that released strains are likely to survive outside of containment.

2. Releases

Estimates of the number of *B. licheniformis* organisms released per production batch are tabulated in Table 1. All calculations are based on use of asporogenic strains with a sporulation deficiency of 10^{-7}. The minimally controlled scenario assumes no treatment of the fermentor off-gas and assumes 100-fold (2 log) reduction of the maximum cell density of the fermentation broth resulting from inactivation (Reilly, 1991). The containment criteria required for the full exemption scenario assume the use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. They also assume an overall 6-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation steps (Reilly, 1991).

TABLE 1. Estimated Number of Viable Bacillus licheniformis Organisms Per Production Batch

Release Media	Minimally Controlled (cfu/day)	Full Exemption (cfu/day)	Release (days/year)
Air Vents	$2x10^{8}$ - $1x10^{11}$	$2x10^{6}$ - $1x10^{9}$	350
Rotary Drum Filter	250	250	350
Surface Water	$7x10^{13}$	$7x10^{9}$	90
Soil/Landfill	$7x10^{15}$	$7x10^{11}$	90

Source: Reilly, 1991

In addition to the releases tabulated in Table 1, spores would be released at a rate of 1.7×10^{10} spores/day in solid wastes and 2×10^{8} spores/day in aqueous wastes (Reilly, 1991). These are "worst-case" estimates which assume that the inactivation procedure against spores is ineffective and the separation efficiency for the rotary drum filter is 99 percent.

3. Air

Specific data which indicate the survivability of *B. licheniformis* in the atmosphere after release are currently unavailable. However, its ability to survive in a broad habitat range and produce spores suggests that this organism would be likely to survive after release. As with naturally-occurring strains, human exposure may occur via inhalation as the organisms are dispersed in the atmosphere attached to dust particles, or lofted through mechanical or air disturbance.

Air releases from fermentor off-gas could potentially result in nonoccupational inhalation exposures due to point source releases. To estimate exposures from this source, the sector averaging form of the Gaussian algorithm described in Turner (1970) was used. For purposes of this assessment, a release height of 3 meters and downward contact at a distance of 100 meters were assumed. Assuming that there is no removal of organisms by additional treatment of off-gases, potential human inhalation dose rates are estimated to range from 3.0×10^{3} to 1.5×10^{6} cfu/year for minimally controlled systems and 3.0×10^{1} to 1.5×10^{4} cfu/year for systems with full exemptions. It should be noted that these estimates represent hypothetical exposures under reasonable worst case conditions (Versar, 1992).

4. Water

The concentrations of *B. licheniformis* in surface water were estimated using stream flow values for water bodies receiving process wastewater discharges from facilities within SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals). The surface water release data (cfu/day) tabulated in Table 1 were divided by the stream flow values to yield a surface water concentration of the organism (cfu/l). The stream flow values for SIC Code 283 were based on discharger location data retrieved from the Industrial Facilities Dischargers (IFD) database on December 5, 1991, and surface water flow data retrieved from the RXGAGE database. Flow values were obtained for water bodies receiving wastewater discharges from 154 indirect (facilities that send their waste to a POTW) and direct dischargers facilities that have a NPDES permit to discharge to surface water). Tenth percentile values indicate flows for smaller rivers within this distribution of 154 receiving water flows and 50th percentile values indicate flows for more average rivers. The flow value expressed as 7Q10 is the lowest flow observed over seven consecutive days during a 10-year period. The use of this methodology to estimate concentrations of *B. licheniformis* in surface water assumes that all of the discharged organisms survive wastewater treatment and that growth is not enhanced by any component of the treatment process. Estimated concentrations of *B. licheniformis* in surface water for minimally controlled and full exemption scenarios are tabulated in Table 2 (Versar, 1992).

TABLE 2. Bacillus licheniformis Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	Q710	Mean	Q710
Minimally Controlled				
10th Percentile	156	5.60	4.5×10^{5}	1.25×10^{7}
50th Percentile	768	68.13	9.11×10^{4}	1.03×10^{6}
Full Exemption				
10th Percentile	156	5.60	4.5×10^{1}	1.25×10^{3}
50th Percentile	768	68.13	9.11×10^{0}	1.03×10^{2}

*MLD = million liters per day
Source: Versar, 1992

The concentrations of *B. licheniformis* spores in surface water were also estimated using the methodology and assumptions described above. Estimated concentrations of *B. licheniformis* spores in surface water are tabulated in Table 3.

TABLE 3. Concentrations of Bacillus licheniformis spores in surface water

Flow	Spores/l	
	Mean	7Q10
l0th Percentile	$1.28x10^{0}$	$3.57x10^{1}$
50th Percentile	$2.60x10^{-1}$	$2.93x10^{0}$

Source: Versar, 1992

5. Soil

The natural habitat for *B. licheniformis* is soil. Therefore, long-term survival in soil may be expected to occur. Human exposures via dermal and ingestion routes, and environmental exposures [i.e., to terrestrial, avian, and aquatic organisms (via runoff)] may occur at the discharge site because of the establishment of *B. licheniformis* within the soil.

6. Summary

Although direct monitoring data are unavailable, worst case estimates using sporulation deficient strains do not suggest high levels of exposure to *B. licheniformis* to either workers or the public resulting from normal fermentation operations.

V. INTEGRATION OF RISK

A. Discussion

Bacillus licheniformis is a ubiquitous, saprophytic, soil bacterium which is thought to contribute to nutrient cycling due to its ability to produce a wide variety of enzymes. This latter feature of the microorganism has been commercially exploited for over a decade. *B. licheniformis* has been used for industrial production of proteases, amylases, antibiotics, and specialty chemicals with no known reports of adverse effects to human health or the environment. The Agency has reviewed three submissions for production of enzymes using genetically modified *B. licheniformis*.

Although the genus *Bacillus* is rather heterogenous based on a wide range of DNA base ratios (32 to 69 mol% G + C), the species *B. licheniformis* is rather homogeneous based on DNA-DNA hybridization studies. Historically, *B. licheniformis* and two closely related species, *B. subtilis*, and *B. pumilus*, were grouped taxonomically into what was known as the subtilis-group. However, recently methods have been developed that allow *B. licheniformis* to be differentiated from these other species.

B. licheniformis is not a frank human pathogen, but has on several occasions been isolated from human infections. Diseases attributed to *B. licheniformis* include bacteremia, opthalmitis following trauma, and there are reports of food poisoning based on circumstantial evidence. However, the literature suggests that there must be immunosuppression of the host, or there must be trauma (especially to the eye) followed by inoculation in high numbers, before infection can occur. *B. licheniformis* does not produce significant quantities of extracellular enzymes or other factors that would predispose it to cause infection. Unlike several other species in the genus, *B. licheniformis* does not produce toxins. Overall, *B. licheniformis* has a low degree of virulence. Although the possibility of human infection is not non-existent, it is low in the industrial setting where highly immunocompromised individuals would not be present. Infection might be a possibility following trauma, but in the industrial setting with the use of proper safety precautions, good laboratory practices, and proper protective clothing and eyewear, the potential for infection of workers should be quite low.

Likewise, the ecological hazards associated with the use of *B. licheniformis* are low. There are various reports in the literature suggesting that *B. licheniformis* is a cause of abortion in livestock. However, Koch's postulates have not been satisfied demonstrating that this microorganism was the causal agent. In most these cases, infections with *B. licheniformis* occurred in animals already in an immunocompromised state resulting from either (1) infection with other organisms or (2) poor nutrition. Apparently, there is immunosuppression associated with maternal and fetal placentas in pregnant livestock, whereby opportunistic microorganisms are capable of causing infection and lesions in fetuses. Although *B. licheniformis* has not been shown to be an etiological agent of livestock abortion, it has been associated with a number of cases. Even so, the association of *B. licheniformis* with livestock abortion is quite small compared to the total number of abortions in livestock caused by all other microorganisms, particularly viruses and fungi.

The use of *B. licheniformis* for industrial production of enzymes should not pose environmental hazards. First, the number of microorganisms released from the fermentation facility is low. In addition, *B. licheniformis* is ubiquitous in the environment, and the releases expected from fermentation facilities operating under the conditions of this exemption will not significantly increase populations of this microorganism in the environment. Therefore, although *B. licheniformis* may be associated with livestock abortions, the use of this microorganism in fermentation facilities will not substantially increase the frequency of this occurrence, even if a scenario for high exposure to *B. licheniformis* released from the fermentation facility to livestock could be envisioned.

In conclusion, the use of *B. licheniformis* in fermentation facilities for production of enzymes or specialty chemicals presents low risk. Although not completely innocuous, *B. licheniformis* presents low risk of adverse effects to human health or the environment.

B. Recommendations

Asporogenic strains of *B. licheniformis*, with a sporulation deficiency of at least 10^{-7}, are recommended for the tiered exemption.

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